HIV Vpr: Roles in Viral Replication and Cellular Metabolism

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Introduction

The auxiliary/non-structural proteins of HIVcan be categorized into 2 groups, essential (Tat and Rev) and accessory (Vpr, Vpx, Vif, Vpu, and Nef). HIV replicates competently *in vitro* in T-cell lines without the need for accessory gene functions (Subbramanian and Cohen, 1994). However, in primary lymphocytes and macrophages, accessory gene products such as Vpr and Vpx contribute to the efficient progression of the virus lifecycle (Balliet et al., 1994; Balotta et al., 1993; Connor et al., 1995; Kwamura et al., 1994; Park and Sodroski, 1995). Furthermore, *in vivo* there must be a selection pressure directed towards preserving these accessory genes since many primary HIV-1 isolates maintain these reading frames open.

Vpr is a 96 amino acid protein that is well-conserved between HIV-1, HIV-2 and SIV. The expression of the mRNA for Vpr is Rev-dependent (Schwartz et al., 1991; Arrigo and Chen, 1991). Vpr is localized to the nucleus of infected cells (Lu et al., 1993), and during late stages of the virus lifecycle is incorporated into the virions at an estimated 5–10 copies per viral particle (Cohen et al., 1990a). Incorporation of Vpr into virion requires the p55Gag precursor and more specifically its carboxy-p6 domain (Lu et al., 1993; Paxton et al., 1993; Zhao et al., 1994a; Kondo et al., 1995). This virion-association of Vpr is unique amongst the HIV accessory proteins and suggests that Vpr may play a significant role soon after virus entry into cells.

Vpr function

A. Viral replication

In some settings, expression of Vpr confers replication efficiency to the virus (Ogawa et al., 1989; Cohen et al., 1990b). This effect is best documented in HIV-1 infections of monocyte/macrophage (Balliet et al., 1994; Connor et al., 1995). In that context, a Vpr(+) HIV-1 replicates 100–1000 folds better than its Vpr(–) counterpart, and mechanistically it has been suggested that Vpr functions at a step after reverse transcription but before proviral transcription (Connor et al., 1995). Likely, this aspect of Vpr function is subserved by cellular protein(s) in established T-cell lines because in these cells both Vpr(+) and Vpr(–) viruses replicate comparably.

B. Nuclear transport of preintegration complexes

HIVs possess the capacity to transport actively viral preintegration nucleic acid-protein complexes into the nucleus of infected cells (Bukrinsky et al., 1992). This unique property accounts for the ability of lentiviruses to infect nondividing, terminally differentiated cells such as microglial cells and macrophages. The nucleophilic property of HIV-1 preintegration complex comes from nuclear localization signals in Gag and Vpr, with either nuclear localization signal being sufficient to target HIV-1 preintegration complexes to the nucleus (Bukrinsky et al., 1993; Heinzinger et al., 1994). However, recently the contribution of Vpr to this process has been disputed (Freed et al., 1995), and a definition of a nuclear localization domain within Vpr is yet imprecisely agreed upon (see Activity domain, below).

C. Transactivation function

It was reported previously that Vpr weakly increases the apparent activity of a variety of promoters in mammalian cells, including the HIV-1 LTR, to a level several folds over background (Cohen et al.,

1990b). Mutational analysis of the HIV-1 LTR was not successful at identifying a Vpr-responsive cis-DNA-element. A recent study using two-hybrid fusion proteins in yeast failed to document an independent activation domain in Vpr (BouHamdan et al., 1996) suggesting that the observed weak activation mediated by Vpr in mammalian cells might be an indirect effect that requires mammalian cell cofactors.

Two recent studies have documented that soluble, diffusible, extracellular Vpr functions to activate virus expression from latently infected cells (Levy et al., 1994; Levy et al., 1995). The findings suggest that treatment of cells with soluble Vpr increased cellular permissiveness to viral replication. One explanation for this phenomenon could be an inductive effect of Vpr on the expression of appropriate cellular genes.

D. Effects of Vpr on the host cell

a. Cell proliferation and killing.

It has been suggested that Vpr plays a role in cell killing and cell proliferation. Biologically, HIV-1 with intact *vpr* is unable to sustain a chronic infection, implying that cells infected with a Vpr(+) virus do not survive (Rogel et al., 1995). On the other hand, infection of the same cells with a Vpr(-) HIV-1 will yield a survivor population from which virus is produced chronically. This suggests that Vpr contributes to a cell-killing phenotype that is inconsistent with maintaining a persistent HIV-1 infection of cells. Indeed, *in vivo*, HIV-1 positive individuals carry viral genomes with *vpr* nonsense mutations at a high frequency in their PBMCs (Nakaya et al., 1994). Further evidence that HIV-1 tends to lose Vpr function in long-term cultures is provided by two HIV-1 molecular genomes recovered from chronically infected cells, MFD (Stevenson et al., 1990) and HXB2 (Popovic et al., 1984). Each is prematurely truncated in Vpr at amino acids 72 and 42, respectively. However, one notes that loss of Vpr function probably is insufficient to affect absolute disease outcome since macaques infected with a Vpr(–) SIV, nevertheless, progress to AIDS (Gibbs et al., 1995). How SIV Vpx contributes to this disease progression remains incompletely elucidated.

A study in yeast has attributed the cytopathic effect of Vpr to a short HS/FRIG motif (see Activity Domain, below). This motif, in peptide form, interferes with membrane ion channel function resulting in cell growth arrest and gross cellular enlargement of yeast. This observed effect of Vpr in yeast can be reversed by prior treatment with calcium channel blockers (Macreadie et al., 1995).

b. Cell differentiation and cycling. In some tumor cells such as human rhabdomyosarcoma cells (TE671 and RD) and osteosarcoma cells (D17), expression of HIV-1 gene products halt cellular proliferation and induce differentiation (Levy et al, 1993). Further dissection of this phenomenon revealed that Vpr alone sufficiently mediated this effect. One explanation for the effect of Vpr on cellular differentiation might relate to its influence on cell cycle progression. Cells expressing Vpr are poised at G2/M and are unable to proceed through mitosis (Rogel et al., 1995; Jowelt et al., 1995). A C-terminal domain within Vpr has been proposed to be the critical element mediating this block in cell cycle progression (DiMarzio et al., 1995). It was further found that cells expressing Vpr had low cyclin B-associated protein kinase activity (Re et al., 1995). Specifically, cyclin B-associated p34cdc2 was found to be in an inactive tyrosine 15 phosphorylated form (He et al., 1995).

c. Fidelity of reverse transcription In a single-cycle infection model, Vpr reduced the error rate of HIV-1 reverse transcriptase 2 fold (Mansky and Temin, 1995). Incorporation of Vpr into virion is required to effect this change in the fidelity of reverse transcription.

d. Association of Vpr with cellular proteins Three cellular proteins have been reported to form protein-protein complexes with Vpr. These are a 41 kDa cytosolic protein that also complexes with glucocorticoid receptor protein (Refaeli et al., 1995), a 180 kDa protein (Zhao et al., 1994), and the cellular uracil DNA glycosylase DNA repair enzyme (UNG; BouHamdan et al., 1996). In the last two cases, amino acids in Vpr spanning positions 60 to 81 and 15 to 77, respectively, were reported to be important for binding to cellular proteins. A firm understanding of the role for Vpr-cellular protein association is yet missing.

Activity domains in Vpr

Vpr has 96 amino acids with an apparent MW of 14 kDa. Several demarcations within Vpr have been proposed (Figure 1). The N-terminal 42 amino acids have been shown to be the oligomerization domain responsible for Vpr hexamer formation (Zhao et al., 1994b; Wang et al., 1995). Within this region, amino acids 16–34 potentially form an amphipathic α helix (DiMarzio et al., 1995; Yao et al., 1995; Mahalingam and Srinivasan, 1995). Mutations in this region prevent Vpr incorporation into virion, but nuclear localization of Vpr in cells is unaffected.

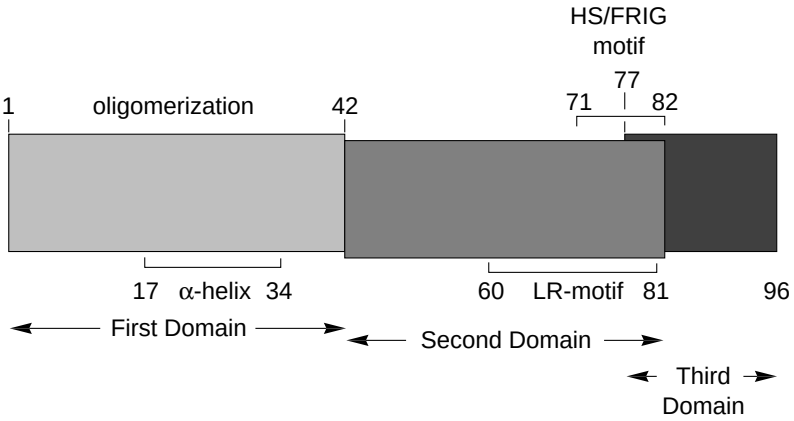

Figure 1. Domain classifications of Vpr protein. The first domain includes amino acids 1–42, the second domain extends from amino acids 42–82, and the third domain covers amino acids 77–96.

A second less-well studied domain extends from amino acids 43 to 82. This region encompasses an HS/FRIG motif (residues 71–82) which is conserved in Sac1p, a yeast protein with cytoskeleton-related function (Macreadie et al., 1995). Another motif in this region is a leucine/isoleucine-rich sequence (LR-motif from amino acids 60–81) which is thought to form a leucine zipper-like structure. The LR-motif is proposed to mediate direct interaction with an 180 kDa cellular protein (Zhao et al., 1994a). Interaction with 180 kDa protein may be important for nuclear localization of Vpr (Zhao et al., 1994a). Other features within this region include a conserved dipeptide (GC; residues 75 and 76) and a potential α-helical motif (residues 46–74). This 46–74 α-helical stretch has been postulated to play a role in virion incorporation and in the stability of Vpr (Mahalingam and Srinivasan, 1995).

The C-terminus of Vpr, residues 77–96, contains 7 arginine residues. This highly charged domain has been suggested to be a nuclear localization signal for Vpr (Lu et al., 1993). Mutations in this region resulted in Vpr proteins that were excluded from the nucleus; furthermore, attachment of the C-terminal sequence of Vpr (residues 77–96) to a cytosolic protein, β-galactosidase, redirected the chimera into the nucleus (Lu and Ratner, 1995). However, within the context of the native Vpr protein, the function of this nuclear localization domain is controversial. Several other mutagenesis studies have failed to link this basic sequence with nuclear localization (Zhao et al., 1994a; Yao et al., 1995; Mahalingam and Srinivasan, 1995). Instead, these investigators found that nuclear localization was lost when the first two N-terminal domains of Vpr were mutated; they proposed instead that the carboxy-domain relates simply to Vpr stability.

A separate study does clearly provide evidence that perturbation of cell cycle progression (see Cell cycle progression, above) is mediated through the C-terminal portion of Vpr (DiMarzio et al., 1995).

Table I lists some engineered mutations in Vpr and their consequential phenotypes. The results in Table I are summarized from 5 studies (Zhao et al., 1994a; Zhao et al., 1994b; Paxton et al., 1993; Lu et al., 1993; Lu et al., 1995). Mutations in the first two domains adversely affect virion incorporation and nuclear localization of Vpr; however, mutations in the third domain are less clearcut and exhibit inconsistent phenotypes.

Table I. Mutations in Vpr

Original Amino Acids and their position(s)	Mutant Amino Acids	Oligomer-ization	Virion incorporation	Nuclear localization	Stability
R32	A		++		++
Δ36–76		–			+
R62	A		++		++
R73	A		++		++
C76	A				–
R80	N			–	++
R85	N			–	++
R87	N			–	++
R88	A		++		++
R88	N			–	++
R90	N			–	++
R87RTR	SATS			++	++
L60IRILQQLLFIHFRI	HARAHQQAAFNHERN			+	
Δ73–96			–		–
Δ78–96				±	+
Δ78–87				++	++
Δ84–96			±		+
Δ88–96				++	++
Δ85–88				±	++
Δ79–82				+	++

*++: wild-type activity; +: mild impairment; ±: moderate impairment; –: severe impairment.

HIV-2 Vpx

Primate lentiviruses consist of HIV-1, HIV-2 and SIV. Based on sequence homology from *gag* and *pol* these groups can be subdivided into i) HIV-1 (including SIVcpz), ii) HIV-2 (including SIVsmm, SIVmac), iii) SIVagm, and iv) SIVmnd. An early classification suggested that subgroups i), ii) and iv) encode *vpr* while subgroups ii) and iii) encode *vpx*, a gene closely related to *vpr*. Recent reanalysis suggests that it is perhaps more appropriate to reclassify all four subgroups as *vpr* containing viruses, and that in subgroup ii) *vpx* likely emerged evolutionarily as a duplication of *vpr* (Tristem et al., 1992).

Vpx has been less extensively studied than Vpr. However, the evidence is that it functions and behaves like Vpr. For instance, Vpx is also required for optimal replication of HIV-2 in PBMCs/macrophages (Kawamura et al., 1994; Park and Sodroski, 1995). It is localized to the virion (Yu et al., 1993), and is packaged by association with Gag (Liska et al., 1994; Wu et al., 1994; Horton et al., 1994). In terms of development of AIDS in macaques, Vpx can, in instances, substitute for Vpr (Gibbs et al., 1994). Hence, it might be appropriate to regard *vpx* as a functionally redundant *vpr* isotype that exists in a subpopulation of primate lentiviruses (Myers et al., 1992).

Concluding Comments

As we unravel the biology of HIVs, it is clear that accessory genes play crucial roles in HIV pathogenesis. To date, several functions have been attributed to Vpr. However, the biological significance of these findings is not completely understood. Also, it would be interesting and vital to understand the mechanisms underlying the functions alluded above.

One word of caution—the nomenclature for genes/proteins in related viruses can be confounding. Whether one calls an entity a Vpr, a Vpx or a Tat can denote and connote different meanings. In hindsight, SIVagm Vpx is probably better named as SIVagm Vpr. Similarly, the *tat* genes from CAEV and related viruses are probably HIV Vpr-equivalents, not HIV Tat-equivalents (Myers et al., 1992). Indeed, recent functional studies have shown that CAEV *tat* is a non-essential gene for virus replication in cells (Harmache et al., 1995), a finding quite distinct from the strict requirement for HIV *tat* in virus replication (see references cite in Huang et al., 1994).

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